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Attraction and feeding responses of Mediterranean fruit fly and a natural enemy to protein baits laced with two novel toxins, phloxine B and spinosad[a]

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Abstract

Studies were conducted to determine attraction and feeding propensity of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), to different protein bait mixtures with and without the insecticides malathion, spinosad, and phloxine B. Protein baits were more attractive to females than to males. Protein-starved females responded more than protein-fed females. The type of protein (USB® yeast hydrolysate enzymatic, Mazoferm®E802, Nu-Lure®Insect Bait, or Provesta® 621 autolyzed yeast extract) in the bait had a major influence on *C. capitata* attraction, which was strongest to fresh Provesta. Aged baits (four day-old) were not as attractive as fresh baits. In feeding propensity studies, highest response was observed for USB protein. On the basis of attraction and feeding responses Provesta (attraction and feeding) and USB (feeding) outperformed the standard Nu-Lure. Protein-starved flies were much more likely to feed on protein compared to protein-fed flies. For protein-starved flies, a mixture of Provesta and malathion repelled fruit flies, compared to a mixture of Provesta and spinosad or phloxine B. This was not the case with protein–fed flies. The wasp *Fopius arisanus* (Sonan), one of *C. capitata*'s primary natural enemies in Hawaii, would not consume protein baits. Our studies suggest that spinosad or phloxine B, with low contact toxicity, mixed with protein baits offers a more environmentally friendly choice for control of *C. capitata* and conservation of *F. arisanus*, whereby the nontarget effects of broad spectrum contact poisons such as malathion can be avoided. Presumably, due to greater selectivity with spinosad and phloxine B bait treatments, the host would be killed, but not the natural enemy.

Introduction

Fruit flies (Diptera: Tephritidae) are among the most economically important pests attacking soft fruits worldwide (White & Elson-Harris, 1992). One of the most notorious species is the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), with a host range that includes more than 350 species of fruits and vegetables (Liquido et al., 1991). Costs to exclude it from areas such as California have totaled almost $500 million during the past 25 years (Carey, 1991, 2000). During the twentieth century, protein baits with insecticides have been one of the most popular and effective methods for control of accidental *C. capitata* introductions and outbreaks. For example, as many as 20 applications of bait sprays were made by air over a 154 km^2 area of California in 1981 to suppress *C. capitata* (Troestschler, 1983). In 1995, eradication of *C. capitata* was achieved in Camarillo, California (Dowell & Penrose, 1995) and in 1997 in Florida (USDA, 1998), with malathion bait sprays.

[a]This article reports the results of research only. Mention of proprietary product does not constitute an endorsement or recommendation by the USDA.

Baits are added to sprays to reduce the proportion of crop or land area covered with spray droplets compared with application of pesticide alone in conventional sprays (Prokopy et al., 1992). Enzymatic protein hydrolysate baits were first used in Hawaii for control of oriental fruit fly, *Bactrocera dorsalis* (Hendel), while malathion became the organophosphate insecticide of choice due to its low mammalian toxicity, low price, and low levels of fruit fly resistance (Steiner, 1952; Steiner et al., 1961; Roessler, 1989). After testing many bait spray formulations in the 1960s, one particular formulation for aerial application was adopted and remains the standard today for *C. capitata* control. It consists of three to four parts Staley's® Protein Insect Bait 7(PIB-7) plus one part of 91 or 95% malathion ULVC (Roessler, 1989). Protein Insect Bait-7 (a combination of corn protein hydrolysate and corn steep liquor) or a similar product (i.e., Nu-Lure® Insect Bait) continues to be the bait of choice owing to its comparative availability, low cost, favorable physical properties, and greater attractiveness to *C. capitata* than most other protein hydrolysates (Steiner, 1969; Roessler, 1989). Similarly, USB® yeast hydrolysate enzymatic is a superior protein product often fed to *C. capitata* adult flies during mass production of fruit flies for sterile insect programs (Vargas, 1989).

Prokopy et al. (1992) pointed out that, although the use of protein bait sprays is widespread, little research has been conducted on behavioral responses of *C. capitata* to bait spray droplets. In field cage studies, these researchers found that PIB-7 baits alone were indeed attractive and phagostimulatory to protein-starved *C. capitata*. Although the addition of malathion to PIB-7 bait did not affect attraction, it did deter feeding. Harris et al. (1971) examined mortality of three species of fruit flies attracted to bait sprays containing malathion or naled and found that only 25% of *C. capitata* attracted to malathion and PIB-7 baits died on site, while the rest flew away.

Overuse of organosphosphate insecticides has been implicated in secondary pest outbreaks, negative effects on beneficial insects, environmental contamination, and adverse effects on human health (Carson, 1962; Hoy & Dahlsten, 1984; Emden & Peakall, 1996). Consequently, replacements for these compounds have been sought. Spinosad, an insecticide derived from the metabolites of the soil bacterium, *Saccharopolyspora spinosa*, has shown outstanding efficacy against target insect pests, comparable to many organophosphate and carbamate insecticides (Sparks et al., 1998; DowElanco, 1994). Most importantly, spinosad demonstrates lower mammalian and environmental toxicity with reduced risk to humans and wildlife than traditional insecticides (DowElanco, 1994). phloxine B is a photoactive dye effective against a variety of insects (Heitz, 1995). When an insect ingests the dye and is exposed to light, the dye oxidizes within the insect's tissues and causes death. It has no contact toxicity against fruit flies and is considered to have little impact on beneficial insects (Dowell, 1997).

Here we report results of behavioral studies of attraction and feeding responses of *C. capitata* to protein baits containing malathion, spinosad, or phloxine B that complemented insecticide trials in coffee fields (Peck & McQuate, 2000; Vargas et al., 2001). We observed behavioral responses of *C. capitata* to Nu-Lure and alternative protein baits, both with and without the novel toxicants, phloxine B and spinosad. We divided our study of *C. capitata* behavior into local attraction experiments conducted in field cages and direct feeding behavior experiments conducted by observing individual flies in the laboratory. In addition, we documented the feeding response of *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), the most important natural enemy of *C. capitata* in Hawaii (Vargas et al., 1995, 2001), to various protein baits.

Materials and methods

Local attraction to protein baits. We tested laboratory-reared *C. capitata* from a colony reared for 110 generations at the USDA, ARS United States Pacific Basin Agricultural Research Center (USPBARC) Insect Rearing Unit in Honolulu (Vargas, 1989). Flies were shipped as pupae to the USPBARC facility in Hilo. Four hundred pupae were placed in large plastic tubs ($32 \times 58 \times 49$ cm) until eclosion. Both protein-fed and protein-starved flies were used in tests. Protein-fed flies were provided with a diet (3:1 by volume) of sugar and USB® enzymatic yeast hydrolysate (United States Biochemical, Cleveland, Ohio), while protein-deprived flies were fed a diet of sugar only. Flies in each group or category were provided water ad libitum and held in a room maintained at 22 ± 5 °C, ambient (40–90%) r. h., and a L12:D12 photoperiod. Flies were tested when they were 10 days old.

All field cage tests were conducted at the University of Hawaii research station at Kainaliu, Hawaii. Trials were conducted inside nylon screen field cages

(3 m tall × 3 m diam.) set up under the roof of an open-air shade house (Prokopy & Vargas, 1996). Four evenly spaced field cages were erected along a North -South transect and numbered sequentially from 1 to 4. Five potted guava (*Psidium guajava* L.) trees were arranged inside each cage to provide a plant canopy 1.25 m in diameter. Initially, cages 1 and 3 contained protein-starved flies, while cages 2 and 4 contained protein-fed flies. Each day a different fly type was assigned to a different field cage to control for any positional effects. At 0900 and 1300 h each day, approximately 300 *C. capitata* flies were released from holding boxes inside each cage at the base of the guava trees to provide a constant number of responding male and female flies within the guava canopy during tests. In experiments with Provesta and pesticides, more insects were available and 600 *C. capitata* flies were released from holding boxes. Cages and guava trees were sprayed with water before each trial to insure that flies were not attracted to baits only because they were thirsty. Testing began at 0930 and ended at 1600 h. After the last test of the day, flies were flushed from canopies and removed from cages.

Test substances were applied as small droplets (ca. 10 μl) from disposable stirring rods to the surface of strips (2 × 5 cm) of coffee (*Coffea arabica* L.) leaves. Coffee leaves were cut to fit inside Petri dishes. Trimming coffee leaves did not increase their attractiveness to *C. capitata* flies (Prokopy et al., 1997). Two strips with 15 drops each were placed inside each glass Petri dish (9 cm diam. × 1.5 cm tall), which was sealed with a tight-fitting screen top. Test materials were applied to leaves 5 min before testing (except for aged lures) and were replenished after two replications of treatments.

Petri dishes with test substances were hung randomly in one of five positions around the perimeter of the canopy within a field cage at the start of each trial. Thin copper wire was attached to screen lids and twisted into a hook to suspend Petri dishes containing baits from wire loops in the guava tree canopy. An observer in the cage slowly circled the perimeter and recorded the number of male and female flies arriving on the top of each dish during a 5 min period. Arriving flies were removed from dishes with a mouth aspirator. After this period, Petri dishes were rotated clockwise one position and recording resumed for another 5 min. This was repeated until each treatment occupied all the positions at the end of 25 min, constituting one replication. For attraction data, numbers of arriving male and female flies were summed for each treatment over each 5 min period allotted to each position for a total of 25 min (one replicate).

Attraction experiment 1. In the first experiment, test substances included water as a control, USB® yeast hydrolysate enzymatic, Mazoferm® E802 (Corn Products, Argo, IL, USA), Nu-Lure® Insect Bait (Miller Chemical and Fertilizer, Hanover, PA, USA), and Provesta® 621 autolyzed yeast extract (Integrated Ingredients, Bartlesville, OK, USA). Since densities of the test substances varied, we had to standardize the test materials. Specific densities were determined with a hydrometer. The bait with the highest density, Provesta, was diluted with water by 20, 50, and 80% (specific gravities of 1.192, 1.120, and 1.046 at 15.5 °C, respectively). The other protein baits were diluted with water to these densities. All five treatments were tested together in three separate field cages, one for each dilution, over a period of three days. Sixteen replicate trials were conducted for each set of dilutions. Dilutions were not directly compared to each other in the same cage. Replicate trials of all three dilution treatments were conducted throughout the daily testing period. The starting time of each dilution experiment (20, 50, or 80%) was randomly assigned.

Attraction experiment 2. The second experiment was conducted with Provesta, Mazoferm, or Nu-Lure droplets on leaves aged for four days, fresh Provesta droplets on leaves, and fresh water droplets on leaves to assay residual attraction of baits. Since protein baits are often applied at weekly intervals (Roessler, 1989), we selected a midpoint of four days for aged bait testing. The fresh bait and the starting concentration of the aged baits had a specific gravity of 1.192 (80% concentration). The baits were aged on coffee leaves in a room maintained at 21 ± 4 °C, ambient (50–75%) r.h., and a L12:D12 photoperiod. Six replicates each were obtained from three different cages for a total of 18 replicates.

Attraction experiment 3. The third experiment examined the attractiveness of Provesta after the pesticides spinosad, phloxine B, or malathion were added. Baits were mixed with pesticides at rates comparable to those used for control of *C. capitata* in the field and were identical to those used in concurrent field tests (Peck & McQuate, 2000; Vargas et al., 2001). Spinosad, phloxine B, and malathion were added to lures at 0.01, 0.5, and 20% active ingredient, respectively. In these pesticide trials, in order to avoid inhaling toxic fumes, dishes with flies were carefully removed from wire hooks, carried to the tent door, and while flies displayed an arrested behavior, were gently brushed outside. Ten replicates each were obtained from each of four different cages for a total of 40 replicates.

Feeding studies. The *C. capitata* used in feeding tests were wild female flies that had emerged from coffee fruits collected in the field. The *F. arisanus* wasps were males and females from colonies maintained at the USDA, ARS, USPBARC Insect Rearing Unit in Honolulu. Flies and wasps were held in 26 × 26 × 26 cm cages upon eclosion. All *C. capitata* were caged as cohorts, which eclosed two days apart. All flies were held at a density of approximately 100 male and 100 female flies per cage, with sucrose and honey provided as a carbohydrate source. Half of the flies received USB protein hydrolysate. *Fopius arisanus* were provided honey. All insects were held in a room maintained at 24 ± 2 °C, 75–90% r.h., and a L12:D12 (L:D) photoperiod.

To test the propensity of female *C. capitata* to feed on different protein baits, we first cut a fresh coffee leaf into a 3 × 3 m square and then placed one drop (2–3 mm diam.) of test substance in the center of the leaf. Each leaf square was placed on an overturned plastic cup (4.5 cm high). The overturned plastic cup was positioned in the center of a cage (26 × 26 × 26 cm), which had one side open for access. The cage was placed on a laboratory bench. Light was provided by two 120 cm, 40-Watt fluorescent bulbs in a light fixture suspended one m above the laboratory bench. Temperature and relative humidity ranged from 22 ± 4 °C, and 70–80%, respectively. Test substances used in the different feeding trials were the same as those used in the local attraction trials. All baits were used at a concentration of 1.192 (specific gravity). Water was used as a control. Baits were mixed with pesticides at rates comparable to those used for control of *C. capitata* in the field (Peck & McQuate, 2000; Vargas et al., 2001). Spinosad, phloxine B, and malathion were added to lures at 0.01, 0.5, and 20% active ingredient, respectively. Tests containing pesticides were conducted on a bench outside the laboratory where temperature and relative humidity averaged 24 ± 2 °C, and 70 ± 10%, respectively. Stock solutions were made for each treatment mixture and these solutions were kept refrigerated at 5 °C between trials.

Female flies were captured randomly from a holding cage using a clear plastic specimen cup (4 cm high × 4 cm diam.). Flies were transferred using the end of a probe to nudge each fly from the cup edge onto the upper leaf surface. Each female was given a maximum of 600 s on a leaf for each trial. A trial ended when a fly stayed on the leaf for 600 s, flew away, or crawled off onto the overturned cup. Flies that left before a minimum of 10 s, were assumed to be in an agitated state, and disqualified (< 5%). When feeding occurred on droplets, time spent feeding was recorded. Total feeding time on a treatment was calculated. Individual flies were tested only once. In our feeding trials one replicate test consisted of testing one different individual *C. capitata* female on each treatment. Once a fly was tested for one treatment it was never used again. In tests of protein baits 25 individual females were tested on each treatment. In trials with baits containing pesticides, there were only enough insects to use 20 females for each treatment. The treatments were presented to the flies in random order within each replicate test. Individual treatments were kept away from the testing areas when not in use. Data were recorded with a stopwatch, paper, and pencil.

To test the propensity of *F. arisanus* to feed on protein baits, we used a protocol similar to that used to test *C. capitata* feeding. We tested the propensity of male and female wasps to feed on Provesta, Nu-Lure, Mazoferm, and USB. In all trials, controls of both honey and water were used. Water was used to control for water present in the baits, while honey was used to show that the insects would feed on a suitable food. We placed leaf squares at a 75° angle and parasitoids were released beneath the drops of test substances. They displayed a negative geotaxis, causing them to move up the leaf and encounter the droplet. When feeding occurred, time spent feeding was recorded. Total feeding time on a treatment was calculated. In our feeding trials, a replicate was considered complete when we had tested one different individual wasp on each treatment. After an individual wasp had been tested once on a treatment, it was not used again.

Statistical analysis. For attraction experiments 1 and 3, a split-plot design was used with main plots arranged in a completely randomized design. The main plot treatments were concentration and state and the subplot factors within a cage were treatments and sex. A GLIMMIX.SAS macro was used. It allowed for Poisson distributed count data as the response and fits a split-plot, mixed model (Littell et al., 1996).

Table 1. Number of *C. capitata* arriving at bait stations[a]

Experiment 1	Sex	Concentration or treatment	Estimate	Lower	Upper
Conc. × sex	F	20%	2.87a	2.17	3.78
	F	50%	3.68a	2.81	4.83
	F	80%	2.99a	2.25	3.98
	M	20%	0.91a	0.63	1.31
	M	50%	0.61a	0.41	0.92
	M	80%	0.66a	0.44	1.01
Treat. × sex	F	Provesta	5.93a	4.90	7.16
	F	Mazoferm	3.98b	3.22	4.93
	F	USB	3.54b	2.83	4.41
	F	Nu-Lure	3.18b	2.53	3.99
	F	Water	1.19c	0.86	1.65
	M	Mazoferm	0.88a	0.60	1.29
	M	Provesta	0.85a	0.56	1.28
	M	Nu-Lure	0.82a	0.55	1.21
	M	USB	0.65a	0.39	1.09
	M	Water	0.48a	0.29	0.79

[a]Concentration × sex and treatment by sex least squares means and 95% confidence intervals. Values in each category followed by the same letter are not significantly different at the 0.0025 level (Littell et al., 1996).

In attraction experiment 2, a randomized complete block was used with the treatment × sex combinations represented in each block/cage. The GLIMMIX.SAS macro was used again with the log link function for count data. For experiment 1, results are presented as concentration × sex and treatment × sex, least squares means, and 95% confidence intervals, which have been transformed back to the original scale of measurement. For attraction experiment 2, results are presented as treatment × sex, least square means, and 95% confidence intervals that have been transformed back to the original scale of measurement (i.e., counts). Finally, for experiment 3, results are presented as treatment × state and state × sex, least squares means, and 95% confidence intervals in the original scale of measurement. Type I error probabilities were controlled at ≤ 0.05 by the Bonferroni method.

For *C. capitata* feeding studies, a completely randomized design was used for selection of flies and treatments. A replicate was considered complete when individual insects had been tested with each treatment. For each experiment feeding response data were subjected to a one way analysis of variance and means were compared using the least significant difference test at a $P = 0.05$ level of significance (Proc GLM, LSD Test, SAS, 1987). Type I error probabilities for treatments were controlled at ≤ 0.05. For *F. arisanus* feeding studies, a two-way layout for sex and treatment was used. A replicate was considered complete when individual insects had been tested with each treatment. For each experiment feeding response data were subjected to a two-way analysis of variance and means were compared using the least significant difference test at a $P = 0.05$ level of significance (Proc GLM, LSD Test, SAS, 1987). Type I error probabilities for treatments were controlled at ≤ 0.05.

Results

In the first attraction experiment, the main effects of protein concentration ($F = 0.21$, $df = 2$, 74.4; $P = 0.8120$) and fly physiological state ($F = 1.43$, $df = 1$, 74.7; $P = 0.2353$) were not significant. Effects of sex ($F = 191.96$, $df = 1$, 381; $P < 0.0001$) and protein treatment ($F = 9.76$, $df = 4$, 381; $P < 0.0001$) were significant. Of the interaction terms, only concentration × sex ($F = 3.31$, $df = 2$, 381; $P = 0.0376$) and treatment x sex ($F = 2.36$, $df = 4$, 381; $P = 0.0528$) were significant. For concentration × sex no least significance difference comparisons among concentration within sex were significant ($P > 0.0025$) (Table 1). Means suggested that responses across concentrations for females versus

Table 2. Number of *C. capitata* arriving at bait stations[a]

Experiment 2	Sex	Treatment	Estimate	Lower	Upper
Treat. × sex	F	Provesta	6.28a	4.05	9.74
	F	4-day-old Provesta	2.35b	1.45	3.80
	F	Water	0.92c	0.51	1.64
	F	4-day-old Mazoferm	0.82c	0.45	1.49
	F	4-day-old Nu-Lure	0.77c	0.41	1.41
	M	Provesta	1.28a	0.75	2.18
	M	4-day-old Provesta	0.82a	0.45	1.49
	M	4-day-old Nu-Lure	0.77a	0.41	1.41
	M	4-day-old Mazoferm	0.66a	0.35	1.26
	M	Water	0.66a	0.35	1.26

[a]Treatment by sex least squares means and 95% confidence intervals. Values in each category followed by the same letter are not significantly different at the 0.0025 level (Littell et al., 1996).

males were not linear. For females higher counts were obtained for the middle concentration, for males higher counts were obtained for the lowest concentration. For the treatment × sex interaction with females, Provesta was significantly more attractive than Mazoferm, USB, Nu-Lure, and water. There were no significant differences among Mazoferm, USB, and Nu-Lure, but all three were significantly different from water. For males, there was no significant difference among proteins.

In the second attraction experiment, treatment ($F = 21.22$, $df = 4, 134$; $P < 0.0001$), sex ($F = 21.55$, $df = 1, 134$; $P < 0.0001$), and treatment × sex ($F = 6.37$, $df = 4, 134$; $P < 0.0001$) were significant. For females, fresh Provesta was significantly more attractive than any of the 4-day-old baits, none of which differed significantly from water in attractiveness except for Provesta. For males, there was no significant difference in response to baits (Table 2).

In the third attraction experiment, treatment ($F = 32.03$, $df = 4, 342$; $P < 0.0001$), sex ($F = 120.73$, $df = 1, 342$; $P < 0.0001$), treatment × state ($F = 5.98$, $df = 4, 342$; $P < 0.0001$), and state × sex ($F = 35.60$, $df = 1, 342$; $P < 0.0001$) were significant (Table 3). Physiological state had a significant effect on response to treatment. Attraction of protein-fed flies to baits was not affected by the addition of spinosad, malathion, or phloxine B. However, attraction of protein-starved flies was reduced significantly when malathion was added to the diet but not when phloxine B or spinosad was added. The sex by state interaction can be explained by a greater effect of protein deprivation on the response of females than males to baits.

The physiological state of the fly had a major influence on the outcome of feeding tests (Table 4). Protein-starved flies fed almost five times as long as protein-fed flies on USB protein. Longest feeding times were obtained with USB protein in separate tests with protein-starved or protein-fed flies (experiment 1). When protein-fed flies were used in the first feeding experiment, response to Mazoferm, Provesta, and Nu-Lure was not significantly different than response to water. When protein-starved flies were used in experiments, responses were: USB > Provesta and Mazoferm > Nu-Lure > water. In the second feeding experiment with Provesta, protein-fed flies fed significantly longer on phloxine B- laced baits than on those laced with malathion. There was no significant difference between spinosad and phloxine B. Protein-starved flies fed significantly longer on phloxine B- or spinosad-laced Provesta baits than on those laced with malathion. In the third feeding experiment with Mazoferm, protein-fed flies fed significantly longer on spinosad-laced bait than on those laced with malathion. There was no significant difference between spinosad and phloxine B. Protein-starved flies fed significantly longer on phloxine B-laced than on malathion-or spinosad-laced Mazoferm baits.

In parasitoid feeding experiments there was no significant difference ($F = 0.70$, $df = 1, 119$; $P = 0.4039$) between male (42.53 ± 12.04 s, lsmeans ± s.e.m.) and female (28.27 ± 12.04 s, lsmeans ± s.e.m.) feeding times. The interaction between sex and treatment was not significant ($F = 0.64$, $df = 5, 119$; $P = 0.6717$). However, food type (protein or honey) had a major influence on feeding propensity of *F. arisanus* (Table 5).

Table 3. Number of *C. capitata* arriving at bait stations with insecticides[a]

Experiment 3	Sex	State	Treatment	Estimate	Lower	Upper
Treat. × state		Protein-fed	Provesta	5.42a	3.92	7.49
		Protein-fed	Provesta + Spinosad	5.11a	3.69	7.07
		Protein-fed	Provesta + Malathion	4.18a	2.97	5.88
		Protein-fed	Provesta + phloxine B	3.73a	2.63	5.30
		Protein-fed	Water	1.77c	1.18	2.64
		Protein-starved	Provesta + Spinosad	6.56a	4.80	8.98
		Protein-starved	Provesta	4.66ab	3.36	6.46
		Protein-starved	Provesta + phloxine B	4.38b	3.15	6.09
		Protein-starved	Provesta + Malathion	2.09c	1.43	3.04
		Protein-starved	Water	1.66c	1.11	2.47
State × sex	F	Protein-fed		6.84a	5.11	9.17
	F	Protein-starved		4.07a	3.00	5.53
	M	Protein-starved		2.86a	2.10	3.91
	M	Protein-fed		2.08a	1.50	2.87

[a]Treatment × state and state × sex, least squares means and 95% confidence intervals. Values in each category followed by the same letter are not significantly different at the 0.0025 level (Littell et al., 1996).

Longest feeding times were obtained with honey. Consumption of protein baits was significantly less than that of water.

Discussion

Earlier behavioral studies on *C. capitata* response to bait droplets suggested that protein-deprived flies were most attracted to protein baits, aged protein baits lost their attractiveness to *C. capitata*, and that malathion did not significantly repel *C. capitata* from approaching bait spray droplets but did significantly deter feeding on them (Prokopy et al., 1992). Our study confirmed the importance of the physiological state of *C. capitata* in feeding responses to protein, the unattractiveness of 4-day-old baits, and the reluctance of *C. capitata* to feed on malathion bait droplets. In contrast to findings by Prokopy et al. (1992), that *C. capitata* flies approach malathion-laced protein droplets, our studies suggest that hungry *C. capitata* females are less likely to arrive at bait stations that contain malathion and protein bait than those with bait only.

Our data further suggest that type of protein influenced attraction of flies to the baits, regardless of concentration. We also found that the Provesta protein outperformed the standard Nu-Lure in attractiveness to females. Recent field studies in Hawaii further suggest that Provesta and Mazoferm can be used in bait sprays for suppression of oriental fruit fly in guava orchards (McQuate et al., 1999) and *C. capitata* in coffee fields (Peck & McQuate, 2000; Vargas et al., 2001), respectively. Aged baits, when compared to fresh baits, were unattractive to *C. capitata*. Since attractiveness of bait droplets is short-lived, baits need to be applied at short intervals or other ingredients added to baits to extend the period of attractiveness. Recommended applications of bait sprays at intervals of 7-14 days (Roessler, 1989) may be too long for the protein component to remain attractive. However, it is recognized that the present study did not measure the effects of weather and the possibility of rehydration of baits. Nonetheless, our data clearly indicate that protein baits need to be examined in the context of both attraction and feeding response. For example, Provesta rated very high with respect to attraction, while USB rated very high with respect to feeding. On the basis of both attraction and feeding, only Provesta rated higher than the standard Nu-Lure.

Our feeding and attraction data indicate that both spinosad and phloxine B could potentially replace malathion in protein bait sprays. The present behavioral study also suggests that spinosad and phloxine B may have other advantages over malathion, such as a lack of repellency when fed to hungry *C. capitata*, that would make them good potential replacements for malathion. Our field cage studies further suggest

Table 4. Feeding time of *C. capitata* females on protein baits without and with insecticides[a]

Experiment	Replicates	Physiological state of female	Treatments	Feeding time in s (mean ± s.e.m.)	
1	25	Protein-fed	USB	102.6 ± 39.2	a
			Mazoferm	32.2 ± 12.6	b
			Provesta	19.5 ± 9.3	b
			Water	6.9 ± 2.3	b
			Nu-Lure	2.1 ± 1.7	b
	25	Protein-starved	USB	485.8 ± 36.1	a
			Mazoferm	267.7 ± 44.9	b
			Provesta	247.8 ± 39.6	b
			Nu-Lure	142.2 ± 32.8	c
			Water	28.2 ± 12.3	d
2	20	Protein-fed	Provesta+0.5% phloxine B	12.4 ± 7.5	a
			Provesta+0.01% spinosad	7.2 ± 4.7	ab
			Water	6.9 ± 2.2	ab
			Provesta	1.5 ± 0.6	ab
			Provesta+20% malathion	0.1 ± 0.1	b
	20	Protein-starved	Provesta+0.5% phloxine B	345.7 ± 37.1	a
			Provesta+0.01% spinosad	274.4 ± 30.4	ab
			Provesta	256.0 ± 28.9	b
			Water	21.8 ± 9.7	c
			Provesta+20% malathion	5.5 ± 4.3	c
3	20	Protein-fed	Mazoferm+0.01%spinosad	101.6 ± 41.2	a
			Mazoferm	96.8 ± 38.4	ab
			Mazoferm+0.5%phloxine B	68.5 ± 30.7	abc
			Mazoferm+20%malathion	15.2 ± 9.4	cb
			Water	12.7 ± 10.4	c
	20	Protein-starved	Mazoferm	304.7 ± 47.0	a
			Mazoferm+0.5% phloxine B	279.6 ± 53.4	a
			Mazoferm+0.01% spinosad	128.7 ± 36.7	b
			Mazoferm+20% malathion	52.6 ± 12.7	bc
			Water	10.8 ± 3.9	c

[a]Values in each experiment followed by the same letter are not significantly different at the 0.05 level (Proc GLM, LSD Test, SAS, 1987).

Table 5. Feeding times of male and female *F. arisanus* wasps on protein baits, honey or water[a]

Replicates	Treatments	Feeding in s (mean ± s.e.m.)	
20	Honey	153.0 ± 38.30	a
	Water	58.60 ± 33.00	b
	Mazoferm	1.00 ± 0.07	c
	Provesta	0.55 ± 0.18	c
	Nu-Lure	0.15 ± 0.08	c
	USB	0.05 ± 0.05	c

[a]Values in the column followed by the same letter are not significantly different at the 0.05 level (Proc GLM, LSD Test, SAS, 1987).

that in concurrent field tests of spinosad, phloxine B, and malathion (Vargas et al., 2001), many *C. capitata* females, depending on physiological state, may have avoided entering malathion-treated fields. Furthermore, nontarget effects of spinosad and phloxine B baits compared to malathion baits should be minimal because of the mode of kill for the three toxicants tested (malathion, spinosad, and phloxine B). Malathion kills insects by contact, vapor action or as a stomach poison (Matsumura, 1975). Thus, any insect landing near malathion-containing bait may die, whether it feeds on the bait or not. Because of this, malathion Nu-Lure protein bait sprays are highly effective for *C. capitata* control (Peck & McQuate, 2000; Roessler, 1989) even though we found that Nu-Lure as a bait is not particularly attractive to *C. capitata* flies. On the other hand, spinosad kills primarily by ingestion, with only limited contact kill (DowElanco, 1994). Phloxine B kills entirely by ingestion (Heitz, 1995). Because spinosad and phloxine B kill primarily by ingestion, consideration should be given to substitute protein baits for Nu-Lure, such as Provesta, that rate high in both attraction and feeding responses.

A significant finding in this study is that *F. arisanus*, the most important natural enemy of *C. capitata* and *B. dorsalis* in Hawaii, did not feed on protein baits but did feed on honey. Honeydew (produced by aphids and scale insects) has been identified as a natural food resource for *F. arisanus* (Bosch & Telford, 1965). Bautista et al. (2001) have documented the beneficial effects of honey added to the diet of *F. arisanus*. In recent studies by Vargas et al. (2001), *F. arisanus* populations recolonized fields sprayed with spinosad or phloxine B protein baits more rapidly than those sprayed with malathion protein bait sprays. Presumably, with spinosad and phloxine B bait treatments, the fruit fly would be killed, but not the natural enemy. Potentially, this leaves *F. arisanus* in the field to attack *C. capitata* that may have been sheltered inside host fruit when pesticide applications occurred. Therefore, it would appear that spinosad or phloxine B would be better choices than malathion from a non-target standpoint for control of *C. capitata* and conservation of *F. arisanus*. Future IPM research of fruit flies should emphasize optimization of the protein and toxicants included in bait, so that non-target effects of broad-spectrum contact poisons can be avoided.

Present research in Hawaii is focusing on integration of techniques, such as environmentally acceptable bait sprays combined with natural enemies, for area-wide Integrated Pest Management (IPM) of fruit flies (i.e., Mediterranean fruit fly, oriental fruit fly, melon fly (*Bactrocera cucurbitae* Coquillett), and Malaysian fruit fly [*Bactrocera latifrons* (Hendel)]. Using bait sprays with parasites may also have broader applications in area-wide programs that are underway in southern Mexico and Central America. One of the major breeding sources of *C. capitata* in Central America is coffee with a total area of coffee cultivation in southern Mexico and Central America in 1998 estimated at 1.5 million ha (FAO, 1998). Control options in this area have recently been restricted by a ban in Guatemala on aerial applications of bait sprays containing malathion for fruit fly suppression. Spinosad or phloxine B bait sprays may be a viable alternative to malathion that could be integrated with sterile fly releases. The use of *F. arisanus* as a biological control agent in conjunction with protein bait sprays may enhance the action of these baits in suppressing fruit fly populations.

Finally, protein hydrolysates are commonly used in glass McPhail traps or plastic substitutes for early detection and monitoring of fruit flies (McPhail, 1939; Roessler, 1989). Identification of superior protein hydrolysates would be useful for improving the sensitivity of these traps for early detection of *C. capitata*. For example, McQuate et al. (1999) have used the Provesta protein in plastic dome traps to monitor *B. dorsalis* populations in guava orchards. Superior traps may be modified further into bait traps or stations for fruit fly control. However, these methodologies require more field-testing and validation.

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